Nitrogen metabolism of calves inoculated with bovine adenovirus-3 or with infectious bovine rhinotracheitis virus

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SUMMARY

Beef calves were inoculated with bovine adenovirus-3 or infectious bovine rhinotracheitis virus. After inoculation, plasma fibrinogen increased, serum phosphorus decreased, and nitrogen and phosphorus digestibility decreased compared with preinoculation values. Urinary N excretion increased when calves developed rectal temperatures > 39.7 C.

Results indicated that clinical infection of calves with infectious bovine rhinotracheitis virus increases urinary N excretion and reduces N and phosphorus balance, and that clinical and subclinical infections with either virus reduce dietary N digestibility.

Stress and infection have detrimental effects on nutrient status, nutrient metabolism, and food intake in man and other nonruminants. During stress and/or infection, a hypermetabolic state develops, characterized by severe weight loss, increased resting metabolic rate, increased muscle protein catabolism,[1-3] increased excretion of nitrogen, phosphorus, and sulfur,[4] and altered response to insulin and catecholamines.[5,6] The hypermetabolic state is mediated primarily by an increased release of catecholamines[5] and is proportional to the severity of stress and/or infection.[3] Numerous changes in blood lipids[7] and amino acids[8] may precede and accompany experimentally induced viral infections.

Feeder calves encounter numerous stressors and infectious agents during marketing and many become morbid or die from bovine respiratory disease. Calves with bovine respiratory disease have low feed intake and lose weight and body condition rapidly. Compared with nonruminants, ruminants have a greater store of nutrients within the digestive tract and have a greater capacity to recycle nutrients such as N. These advantages could aid the ruminant in buffering some of the adverse effects of a hypermetabolic state.

The present study was conducted to evaluate the effects of experimentally induced bovine adenovirus-3 (BAV-3) or infectious bovine rhinotracheitis virus (IBRV) on N metabolism and blood biochemistry values of beef calves.

Materials and Methods

Cattle—Eight Hereford steers (mean weight = 200 kg) were used in 2 experiments. Experiment 1 was performed in December 1982, and experiment 2 was performed in February 1983. The same 8 calves were used in both experiments. All calves were adjusted to a diet of 95.4% barley, 2% molasses, and 2.6% vitamins and minerals. The diet contained 11.5% crude protein, 0.45% calcium, and 0.43% phosphorus on a dry matter basis. Each calf was fed 1.25% of their body weight in 2 equal daily feedings. Water was available ad libitum. Calves were housed in metabolism stalls to facilitate collection of urine and feces.

Experimental design—After a 7-day stall-adjustment period, feces and urine were collected from each calf and were weighed, and a 10% subspecimen of feces and urine was frozen daily for 21 days. Urine was collected in plastic buckets containing 200 ml of 20% hydrochloric acid to prevent ammonia losses. At the end of the first 7-day fecal and urine collection period, subspecimens from each calf were composited and stored at −20 C. Calves were then inoculated (intranasal aerosol) with BAV-3 (10^5 TCID$_{50}$/nostril; experiment 1) or with 2.7×10^5 plaque-forming units (PFU) of IBRV (experiment 2). During the subsequent 14 days, urine and feces subspecimens were composited at 3- or 4-day intervals and stored at −20 C. Urine specimens from each calf were collected and frozen daily.

In experiment 1, blood samples were collected via jugular venipuncture 5 days before inoculation, on the day of inoculation, and on postinoculation days (PID) 3, 6, 9, and 14. In experiment 2, blood samples were collected on the day of inoculation and on PID 3, 7, 9, and 14. Heparinized blood samples were collected and used for complete blood cell (CBC) counts and fibrinogen concentration determinations. Serum samples were allowed to clot and were separated by centrifugation at 3,000 × g at 10 C. Serum samples were used for biochemical concentration determinations. Serum samples collected immediately before virus inoculation and at PID 14 were serologically evaluated for BAV-3 or IBRV.

Rectal temperatures were recorded daily by use of an electronic thermometer.[a] Each calf was individually weighed (accuracy = ± 1 kg[b]) at the beginning and end of each experiment. Nasal swabs for BAV-3 detection were collected immediately before inoculation and on PID 1, 2, 4, 6, and 14. Adenovirus in nasal secretions was detected in bovine fetal kidney (BFK) tube-cell cultures by detection of cytopathic effect. Nasal swabs for IBRV titration were collected immediately before virus inoculation and on PID 2, 4, 6, 10, and 14. Nasal excretion of IBRV was titrated by use of a plaque method in BFK cell cultures.[9]

Analyses—Serum was analyzed for glucose, urea N, total protein, albumin, calcium, inorganic phosphorus, creatinine, total bilirubin, creatinine phosphokinase, aspartate transaminase,

alanine transaminase, and alkaline phosphatase using automated procedures.[c] Feed, feces, and acidified urine specimens were analyzed for nitrogen, using micro-Kjeldahl.[d] In experiment 2, feed, feces, and urine were analyzed for phosphorus by automated procedures.[e] The CBC counts were determined, using a Coulter counter.[f] Plasma fibrinogen concentrations were determined, using a refractometer.[g]

Viruses—Bovine adenovirus-3, strain 62N was obtained[h] and propagated in BFK cell cultures. The BAV-3 had a concentration of 10^5 TCID$_{50}$/ml and was frozen at -70 C. In experiment 1, frozen 1-ml ampules of BAV-3 were thawed and the undiluted contents of each ampule immediately were inoculated intranasally into each calf (1 ampule/calf). The Cooper strain of IBRV was obtained from the National Animal Disease Laboratory, Ames, Iowa, and was used in experiment 2. The IBRV was supplied in 1-ml ampules containing 2.7×10^7 PFU of IBRV and was stored at -70 C. Each ampule of virus was thawed. Immediately after thawing, the contents of each ampule were diluted 1:100 in Eagle's minimum essential medium (MEM) and was given intranasally to each calf (1 ml/nostril). The Colorado I vaccinal strain of IBRV was propagated in BFK cell cultures and used in viral serum neutralization tests.

Neutralization tests—Serum antibody against BAV-3 and IBRV was determined by use of microtitration in complete monolayers of BFK cells, essentially as described previously.[10,11] A serum sample dilution was considered positive for antibody when the dilution of serum completely protected BFK cells from cytopathic effect in at least 3 of 4 Microtiter wells. Before evaluation, sera were inactivated by heating at 56 C for 30 minutes.

Cell cultures—Bovine fetal kidneys obtained at an abattoir were used to prepare BFK cell cultures. Growth medium for the BFK cells consisted of MEM prepared in Hanks's balanced salt solution (0.033% NaHCO$_3$) and 10% bovine fetal serum. Maintenance medium consisted of MEM with 5% bovine fetal serum and 0.067% NaHCO$_3$. Potassium penicillin G, streptomycin sulfate, and amphotericin B were included in all media. Cultures were incubated at 37 C in CO$_2$ (1% to 2%).

Statistical analysis—Data were analyzed by use of analysis of variance as a split plot in time, using the general linear models procedure of the Statistical Analysis System.[12] Day and period effects were analyzed by use of Duncan's multiple range test. Differences were considered statistically significant at $P < 0.05$.

Results

Experiment 1—Seven of 8 calves had at least a 4-fold increase in serum antibody titer against BAV-3 between the day of inoculation and PID 14. All calves excreted BAV-3 by PID 4 (Table 1). The calves did not develop a febrile response to BAV-3 inoculation or show clinical signs of BAV-3 infection.

Total RBC counts decreased from day 0 (preinoculation) to PID 3 (Table 2). After inoculation, plasma fibrinogen increased and serum inorganic phosphorus decreased, compared with preinoculation values. Serum urea N was lower on PID 9 and 14 than at day 0. Other hematologic or biochemical determinants were not affected by BAV-3 inoculation.

[c] SMA 12/60, Technicon Instrument Corp, Tarrytown, NY.

[d] Kjeltech, Tecator Instruments, Hernden, Va.

[e] Autoanalyzer II, Technicon Instrument Corp, Tarrytown, NY.

[f] Coulter Instrument Co Inc, Hialeah, Fla.

[g] American Optical Corp, Buffalo, NY.

[h] Dr. D. Mattson, Oregon State University, Corvallis.

TABLE 1—Serum antibody titers and nasal excretion of bovine adenovirus-3 in calves inoculated with bovine adenovirus-3

Steer No.	Serum titer		Nasal excretion				
	Day 0*	PID 14	PID 1	PID 2	PID 4	PID 6	PID 14
1	4	64	−	−	+	−	−
2	32	32	−	+	+	+	−
3	8	64	+	+	+	+	−
4	8	32	−	+	+	−	−
5	< 4	16	−	+	+	+	−
6	4	32	−	+	+	+	−
7	8	64	−	+	+	−	−
8	4	32	−	+	+	−	−

* Preinoculation (day of inoculation).

PID = postinoculation day; − = no excretion of bovine adenovirus-3; + = excretion of bovine adenovirus-3.

Inoculation with BAV-3 did not affect feed intake, N balance, or urinary N excretion (Table 3). However, N and dry matter digestibilities were significantly lower between PID 6 and 10 than preinoculation.

Experiment 2—Four of the 8 steers developed clinical signs of IBRV infection (ie, high rectal temperature [> 39.7 C], nasal excretion of IBRV [Table 4], and high neutrophil counts [Table 5]). All calves had at least a 4-fold increase in serum antibody titers against IBRV, and 7 of 8 calves excreted IBRV in their nasal secretions. Febrile calves (temperature > 39.7 C; n = 4) had lower geometric mean titers (GMT) of IBRV serum neutralizing antibody (titer < 4) than did nonfebrile calves (mean titer = 9.5; n = 4) at the time of virus inoculation. Febrile calves excreted more virus ($P < 0.10$) than did nonfebrile calves. At PID 2 and 4, febrile calves had GMT of IBRV excretion of 320,000 PFU and 164,000 PFU, whereas nonfebrile calves had GMT of 1,700 PFU and 1,090 PFU. From PID 1 through 3, RBC counts, blood hemoglobin concentrations, PCV, and leukocyte and lymphocyte counts decreased in all steers. In nonfebrile calves, total neutrophil counts decreased from day 0 to PID 3. In febrile calves, neutrophil counts increased from day 0 to PID 3 and decreased from PID 3 to 7, and total eosinophil counts decreased from day 0 to PID 3.

Compared with preinoculation values, viral inoculation significantly decreased serum phosphorus and urea N concentrations in all calves (Table 5) and increased fibrinogen concentrations in febrile calves, but not in nonfebrile calves. Other blood or serum values were not significantly affected by IBRV inoculation.

During the first 7 days after IBRV inoculation, febrile calves had a lower N digestion and N balance than did nonfebrile calves (Table 6). The lower N balance was a result of an increase in fecal and urinary N excretion. In general, increased urinary N excretion coincided with increased rectal temperature (Fig 1). The increased urinary N excretion lasted for about 3 days (the same length of time as the febrile response).

From PID 1 through 7, febrile calves had a lower phosphorus balance than did nonfebrile calves due to an increased fecal phosphorus excretion (Table 7). Urinary phosphorus accounted for < 20% of the total phosphorus excretion and was not affected by virus inoculation.

Discussion

Seven of the 8 calves had serum antibody titers to BAV-3 in experiment 1, and 3 of the 8 calves had serum an-

TABLE 2—Mean values of blood and serum determinants affected by inoculation of calves (n = 8) with bovine adenovirus-3

Determinant	Day −5*	Day 0†	Postinoculation day				SEM
			3	6	9	14	
RBC (10⁶/ml)	8.1^{a,b}	8.3^b	7.8^a	8^a	8^a	8.5^b	0.1
Fibrinogen (mg/dl)	120^c	178^c	431^a	341^b	162^c	140^c	20.2
Phosphorus (mg/dl)	7.9^{a,b}	8.2^b	7.5^a	8.2^b	8.1^b	8.2^b	0.1
Urea N (mg/dl)	8.6^a	8^a	7.5^{a,b}	7.9^a	6.9^b	6.9^b	0.3

* Five days before inoculation with bovine adenovirus-3. † Preinoculation (day of inoculation with bovine adenovirus-3).
Values in same row with different lettered superscripts are significantly different (P < 0.05).

TABLE 3—Mean nitrogen metabolism values in 8 calves before and after inoculation with bovine adenovirus-3 (experiment 1)

Determinant	Day −5 to 0	Day 0 to PID 5	PID 6 to 10	PID 10 to 14	SEM
Nitrogen intake (g)	39.9	39.9	39.9	39.9	0.2
N digestion (%)	80.7^c	79.5^{b,c}	75.2^a	77.3^{a,b}	0.8
N balance (g)	10.6	12.2	13.1	15.0	0.9
Urine N (g)	21.6	19.5	16.9	15.8	1.5
Dry matter digestion (%)	85.5^{b,c}	84.4^{b,c}	81.8^a	83.6^b	0.5

Day −5 = 5 days before inoculation with bovine adenovirus-3; day 0 = preinoculation (day of inoculation with bovine adenovirus-3); PID = postinoculation day.
Values in the same row with different lettered superscripts are significantly different (P < 0.05).

TABLE 4—Mean serum titers against infectious bovine rhinotracheitis virus (IBRV) and mean nasal excretion of IBRV in 8 calves after intranasal inoculation with IBRV

Calf No.	Serum titer		Nasal excretion (× 10³ plaque-forming units)			
	Day 0*	PID 14	Day 0	PID 2	PID 4	PID 6
1	32	≥ 128	0	0	0	0
2	16	≥ 128	0	5.2	2.6	0
3†	< 4	≥ 128	0	420	64	0
4	< 4	≥ 128	0	160	54	0
5†	< 4	≥ 128	0	460	80	0
6	8	64	0	10	10	0
7†	< 4	≥ 128	0	100	1,000	0
8†	< 4	≥ 128	0	540	140	0

*Preinoculation (day of inoculation with IBRV); † calf became febrile after inoculation.
PID = postinoculation day.

TABLE 5—Mean hematologic and serum biochemical values in eight calves after inoculation with infectious bovine rhinotracheitis virus

Determinant	Days after inoculation				SEM
	0*	3	7	9	
Phosphorus (mg/dl)	9.1^c	7.4^a	8.6^{b,c}	8.1^b	0.15
Urea N, (mg/dl)	9.6^c	7.6^{a,b}	6.8^a	8.9^{b,c}	0.4
RBC (× 10⁻⁶/mm³)	8.2^b	7.7^a	8.2^b	8.5^b	0.11
Hemoglobin (g/dl)	13.5^b	12.9^a	13.6^b	13.8^b	0.18
PCV (%)	38^b	35^a	38^b	39^b	0.5
WBC (× 10⁻³/mm³)	10.3^{b,c}	8.6^a	9.2^{a,b}	10.6^c	0.4
Lymphocytes (No./mm³)	7,100^{b,c}	5,749^a	6,582^b	7,361^c	238
Neutrophils (No./mm³)					
Nonfebrile calves (n = 4)	2,807^b	1,930^a	2,415^b	2,828^b	141
Febrile calves (n = 4)	2,324^b	3,416^b	1,278^a	2,281^b	217
Eosinophils (No./mm³)					
Nonfebrile calves (n = 4)	589	685	787	712	102
Febrile calves (n = 4)	370^{b,c}	0^a	248^b	184^b	54
Fibrinogen (mg/dl)					
Nonfebrile calves (n = 4)	200	255	191	241	19
Febrile calves (n = 4)	150^a	328^b	302^b	295^b	44

* Preinoculation (day of inoculation with infectious bovine rhinotracheitis virus).
Values in the same row with different lettered superscripts are significantly different (P < 0.05).

TABLE 6—Mean nitrogen metabolism values in eight calves before and after inoculation with infectious bovine rhinotracheitis virus (experiment 2)

Determinant	Day −5 to 0	Day 0 to PID 7	PID 8 to 14	SEM
Dry matter intake (g)	2,679	2,753	2,656	22
Nitrogen intake (g)	49	51	49	0.4
N digestion (%)				
Nonfebrile calves (n = 4)	65.2	63.6	63.0	1.1
Febrile calves (n = 4)	63.5^a	55.1^b	59.5^{a,b}	1.5
N balance (g)				
Nonfebrile calves (n = 4)	12.6	12.0	10.7	0.8
Febrile calves (n = 4)	15.5^a	8.3^b	13.1^{a,b}	1.2

Day −5 = 5 days before inoculation; day 0 = preinoculation (day of inoculation); PID = postinoculation day.
Values in the same row with different lettered superscripts are significantly different (P < 0.05).

tibody titers to IBRV in experiment 2 at the time of inoculation. In experiment 1, serum antibody to BAV-3 may have protected calves from development of severe clinical signs of BAV-3 infection, but did not prevent establishment of infection (evidenced by nasal excretion of BAV-3 and increased serum antibody titers). In experiment 2, four calves (all seronegative [titer < 4] to IBRV) became clinically ill (ie, febrile) and the other 4 calves had evidence of subclinical infection (increased serum antibody titers and nasal excretion of IBRV). Viral excretion was minimal after PID 6.

In experiment 2, nonfebrile calves had a significant decrease in total neutrophils on PID 3. In contrast, febrile calves had a slight neutrophilia on PID 3 and a neutropenia on PID 7, indicating that these calves may have had a secondary bacterial infection.[13]

In both experiments, viral inoculation decreased RBC counts from the preinoculation values. However, the decrease was small, and values stayed within the normal range.[13]

In all calves of experiment 1 and in febrile calves of experiment 2, plasma fibrinogen concentrations increased after inoculation. In nonfebrile calves in experiment 2, plasma fibrinogen concentrations generally increased (P < 0.10) after inoculation. These findings agree with reports that even mild infection[14] or stress[15,16] can increase plasma fibrinogen concentrations.

In both experiments, serum P concentrations decreased after inoculation. Similar decreases in serum P have been reported in calves inoculated with IBRV and Pasteurella haemolytica.[14] In the present study, the lower serum P concentrations in calves after inoculation may have resulted from reduced P absorption from the gut (Table 7),
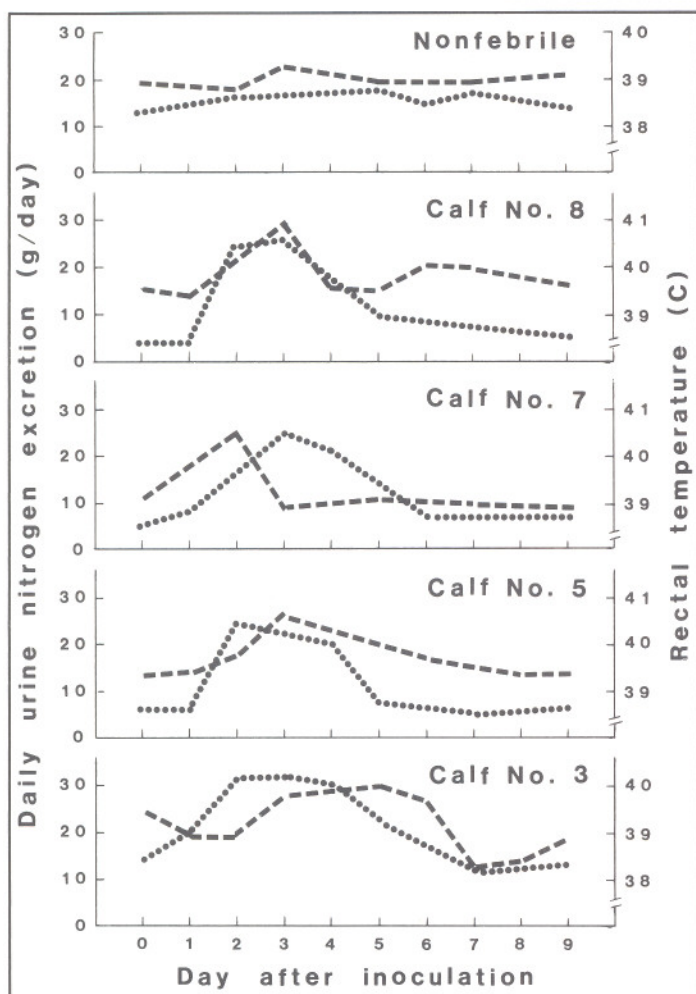
Fig 1—Daily rectal temperature (dotted line) and urinary nitrogen excretion (dashed line) of calves inoculated with infectious bovine rhinotracheitis virus in experiment 2. Mean values are given for the 4 nonfebrile calves. Values are given for each of the 4 febrile calves.

TABLE 7—Mean phosphorus metabolism values of nonfebrile (n = 4) and febrile (n = 4) calves before and after inoculation with infectious bovine rhinotracheitis virus (experiment 2)

Determinant	Day −5 to 0	Day 0 to PID 7	PID 8 to 14	SEM
P intake (g/day)				
Nonfebrile calves (n = 4)	12.5	12.8	12.4	0.31
Febrile calves (n = 4)	12.2	12.3	12.2	0.28
Fecal P (g/day)				
Nonfebrile calves (n = 4)	7.4	7.4[a]	7.4	0.58
Febrile calves (n = 4)	6.6	9.4[b]	8.3	0.63
Urine P (g/day)				
Nonfebrile calves (n = 4)	1.1	1.4	1	0.22
Febrile calves (n = 4)	1.3	1	0.5	0.26
Apparent P digestion (%)				
Nonfebrile calves (n = 4)	40.8	42.1[a]	40.3	1.9
Febrile calves (n = 4)	45.9	23.6[b]	32	2.2
P balance (g/day)				
Nonfebrile calves (n = 4)	4	4.1[a]	4	0.27
Febrile calves (n = 4)	4.2	1.9[b]	3.7	0.62

Day −5 = 5 days before inoculation; day 0 = preinoculation (day of inoculation); PID = postinoculation day.

Values in the same column with different lettered superscripts are significantly different (P < 0.05).

lesterol, and glucose have been reported in calves after inoculation with IBRV and *Pasteurella haemolytica*.[14] Although similar trends were found in calves of the present study, changes in serum values were small and were not significant. These differences may have been due to differences in the severity of the infection and/or due to differences in cause of infection (ie, viral only vs viral and bacterial).

Nitrogen balance and urinary N excretion in the calves were affected only when calves developed a febrile response. However, nitrogen digestibility was reduced in calves in both experiments after inoculation.

Compared with preinoculation values, N digestibility decreased 7% between PID 6 and 10 in experiment 1 and decreased 13% between day 0 and PID 7 in experiment 2. In calves fed a 65% concentrate diet and inoculated with IBRV,[i] N digestibility reportedly decreased 25% between PID 2 and 7. A number of factors could cause a reduced N digestibility during infection, including increased rate of passage of digesta, reduced absorption of N from the gastrointestinal tract, or reduced microbial fermentation in the ruminoreticulum. Increased secretion of thyroxine and triiodothyronine, such as that which occurs during cold stress, increases the rate of passage of digesta and reduces digestibility by 4% to 6%.[17] In man, thyroxine secretion, as measured by protein-bound iodine, may increase during bacterial or viral infection; however, the increase depends on the severity, source, and stage of infection.[18] In subclinical infections, an increased secretion of thyroxine may cause reduced N digestibility.[17] In clinical infections when the decrease in digestibility is greater than in subclinical infections, other factors also may be involved in reducing digestibility. Rumen bacteria grow slowly at temperatures > 40 C.[19] Therefore, during a fe-

reduced P resorption from the tissues, increased P utilization, or a combination of these factors.

Compared with preinoculation values, serum urea N concentrations decreased in both experiments; however, in experiment 1, the decrease was small and did not occur until PID 9. This finding is in contrast to previous results in calves experimentally infected with *Pasteurella haemolytica*[14] or IBRV.[i] Schmitz et al[14] noted a significant decrease in feed intake after inoculation, which may account for at least a portion of the increase in serum urea N concentrations they reported. Orr and Hutcheson[i] found that although feed intake was not depressed, calves with a severe experimentally induced IBRV infection developed a negative N balance and had an increase in serum urea N concentration only on PID 5. Therefore, an increase in serum urea N during infection may be indicative of a negative N balance and increased tissue protein catabolism.

Increases in serum aspartate transaminase, alanine transaminase, and bilirubin and decreases in serum alkaline phosphatase, calcium, total protein, albumin, cho-

[i] Orr CL, Hutcheson DP. The use of a stable isotope of nitrogen to investigate protein turnover in the whole animal (abstr). *J Anim Sci* 1985;61(Suppl 1):461.

brile response, rumen temperature may be high enough to cause decreased rumen fermentation with subsequent reduction in digestibility.

Increasing the rectal temperature of calves to 40 C by housing them at 35 C in environmentally controlled rooms reportedly increased urinary N excretion and muscle tissue catabolism.[20,21] In the present study, however, increased N excretion in febrile calves could not be attributed directly to an increase in rectal temperature because, in one calf, the increase in N excretion occurred before development of fever; in another calf, the increase in N excretion occurred after the febrile response began. In man, alterations in plasma lipids, decreases in plasma amino acids, and increases in serum growth hormone and adrenal glucocorticoids develop shortly before onset of fever.[7,8,22] Therefore, in calves of the present study, the increase in urinary N excretion and the decrease in eosinophils and lymphocytes after viral inoculation may have been attributable to an increased secretion of glucocorticoids.[13]

Results of the present study indicated that clinical IBRV infection in calves can increase urinary N excretion and reduce N and P balance (similar to findings in virus-infected nonruminants) and that clinical and subclinical BAV-3 and IBRV infections can reduce dietary N digestibility in calves.

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